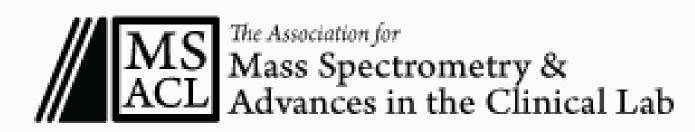
# **Assessment of the Impacts of Blood Collection Tube Types on Blood Acylcarnitine Determinations**



# Background

Blood acylcarnitine profile analysis is a powerful tool to diagnose numerous inherited metabolic disorders, including many mitochondrial fatty acid oxidation disorders and organic acidemias. It is used for follow-up testing of screen positive results from newborn screening programs and in the evaluation of children and adults suspected of having a fatty acid or organic acid disorder. Serum or plasma samples, the latter obtained with a variety of anticoagulants, are normally accepted for acylcarnitine profile analysis. In view of the diverse types of blood collection tubes used in acylcarnitine analyses, it is important to evaluate possible matrix effects on the measurement of the many acylcarnitines that are assessed in blood acylcarnitine assays.

# Study Design

52 acylcarnitines and related analytes were measured by liquid chromatography-tandem mass spectrometry using plasma obtained from sodium heparin (green top tube), lithium heparin (green top tube), and EDTA (lavender top tube) anticoagulated tubes, as well as serum (red top tube). Samples from each blood tube type were obtained at the same time from three healthy individuals. Each sample is assayed in triplicates

# Instrument conditions:

	Compound	Start Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V	)
1	C3:1 ACRYLYL	0.2	3	273	85	23	
2	C3:1 ACRYLYL	0.2	3	273	142	16	
3	butyrobetaine	1	2.5	202.2	87.1	17.5	
4	butyrobetaine	1	2.5	202.2	143	14	
	C41 CROTONU		4	287	85	24	
6	C41 CROTONY	1	4	287	141	16	
,	4-0H-C4	1	1.5	305.2	87	24.9	
8	4.0H.C4	1	3.5	305.2	103	20.8	
•	0	1.5	3.5	261	85	24	
c		1.5	3	261	145	17	
1		1.5	3	263.16	85.1	23	
12		1.7	1.2	305	85	25	
13		1.7	3.2	305	142	17	
4		2.2	3.2	305	85	26	
		2.2	3.7	319	145	19	
5							
6		2.2	3.7	322	85	26	
7		2.4	3.9	275	85	23	
8		2.4	3.9	275	142	16	
		2.4	3.9	276.95	85.1	24.5	
C		3.5	7	333.3	85.1	26	
1		3.5	7	333.3	141.1	18	
2		3.8	5.3	289	85	24	
3		3.8	5.3	289	141	17	
		3.8	5.5	291.25	85.04	22.19	
	5 C4 butyryl	4	5.5	289	85	24	
	6 C4 butyryl	4	5.5	289	141	16	
	7 C5:1 tigloy!	4.8	6.3	300.2	85	23	
	C5:1 tigloyl	4.8	6.3	300.2	141.1	16	
	FIGLU	5	8	287.1	157	21	
	FIGLU	5	8	287.1	213	16	
	C5:1 3-methyl-		6.5	300.2	85	24	
	C5:1 3-methyl-		6.5	300.2	141.2	16	
	C5 2-methyl-bu		7.5	302.2	85.1	24	
	C5 2-methyl-bu		7.5	302-2	141.1	17	
		5.5	7.5	302.3	85.1	24	
ŧ	5 C5 valeryl	5.5	7.5	302.3	141.1	16	
9		5.5	7.5	303.3	85.1	25	
\$	C5 isovaleryl	5.5	7.5	303.3	142.1	17	
5	CS-D9	5.5	7.5	311.3	85.1	22.44	
1	C6:1	6.5	9	314.3	85	25	
1	C6:1	6.5	9	314.3	141	17	
l2	2 C3DC	6.5	9	361	85	27	
13	G3DC	6.5	9	361	246	20	
μ	4 C10:1-OH	7	10	387	85	27	
13	5 C10:1-OH	7	10	387	145	22	
46		7.5	9	317.3	85.1	25	
		7.5	9	317.3	141.2	17	
12		7.5	9	361	85	28	
			-				-
	Compound	Start Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	b
	C14:2	8.8	10.3	424.3	85.1	28	
	C14:2	8.8	10.3	424.3	141.1	20	
	C140H	8.8	10.3	444.4	85.1	31	
	C140H	8.8	10.3	444.4	201.1	19	

98	C14:2	8.8	10.3	424.3	141.1	20	
99	C140H	8.8	10.3	444.4	85.1	31	
100	C140H	8.8	10.3	444.4	201.1	19	
101	C14:1	9	10.5	426.3	85	27	
102	C14:1	9	10.5	426.3	141.1	20	
103	C14-D9	9	10.6	438.43	85.05	28.72	
104	C160H	9	10.5	472.4	85.095	30.74	
105	C160H	9	10.5	472.4	141.071	21.85	
106	C18DC	9	12	570.5	85.1	36	
107	C18DC	9	12	\$70.5	297	24	
108	C14	9.1	10.6	428.4	85.1	30	
109	C14	9.1	10.6	428.4	141.1	19	
110	C18:1-OH	9.3	10.8	498.3	85	31	
111	C18:1-OH	9.3	10.8	498.3	141	24	
112	C16:1	9.5	11	454.4	85.1	28	
113	C16:1	9.5	11	454.4	141.1	21	
114	C16	9.5	11.5	456.3	85.1	30	
115	C16	9.5	11.5	456.3	141	23	
116	C16-D3	9.5	11.5	460.39	85.13	27.37	
117	C18:2	9.5	11	480.45	85.042	28.72	
118	C18:2	9.5	11	480.45	141.125	23.53	
119	C18	9.5	12	484.5	85	29	
120	C18	9.5	12	484.5	141	22	
121	C18-D3	9.5	12	487.5	85	29	
122	С180Н	9.5	11	500.45	85.125	30.99	
123	C180H	9.5	11	500.45	145.054	25.56	
124	C16DC	9.5	11	543.5	85.04	32.7	
125	C16DC	9.5	11	543.5	270	27.4	
126	C18:1	9.8	11.3	482.45	85	29.09	
127	C18:1	9.8	11.3	482.45	141.125	22.14	

# Liquid chromatography conditions

Mobile phase A: 0.1% formic acid (v/v) in Clinical Laboratory Reagent Water (CLRW) Mobile phase B: acetonitrile Column: Kinetex 1.3 µm C18 100Å LC column 50 x 2.1 mm Column temperature: 65°C Injection volume: 10 µL

Start	Len	Flow	Grad	%A	%В	%
0.00	0.50	0.60	Step	98.0	2.0	
0.50	0.50	0.60	Ramp	88.0	12.0	
1.00	6.00	0.60	Ramp	78.0	22.0	
7.00	3.50	0.60	Ramp	15.0	85.0	
10.50	1.00	0.60	Step	2.0	98.0	
11.50	3.50	0.60	Step	98.0	2.0	

Dahai Shao, PhD, Lidong Zhai, PhD, Richard Giles, PhD, Kathleen Pap, Marvin Natowicz, MD, PhD Department of Laboratory Medicine, Robert J. Tomsich Pathology & Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH

# Methods & Results

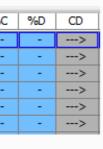
Mass spectrometry transitions & parameters

Dwell Time (ms)	RF Lens (V)
	50
	50
	41
	41
	55
	55
	65
	65
	51
	51
	49
	58
	58
	61
	61
	62
	48
	48
	40
	4/
	62
	57
	57
	63
	63
	55
	55
	58
	58
	55
	55
	55
	60
	60
	57
	57
	58
	58
	30
	59
	59
	100
	100
	74
	74
	59
	59
	70
	70
	74 51 51
well Time (ms)	
	79
	79
	77

	Compound	Start Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Min Dwell Time (ms)	RF Lens (V
19	свон	7.5	9	361	141	19		70
50	C4DC succinyl	7.5	9	375	85	27		72
51	C4DC succinyl	7.5	9	375	101	27		72
52	C4DC methylma	7.5	9	375	85	27		77
53	C4DC methylma	7.5	9	375	101	30		77
54	C5DC glutaryl	7.5	9	389.3	85.1	29		70
55	CSDC glutaryl	7.5	9	389.3	115	27		70
56	C5DC-D3	7.5	9	392	85	28		74
57	67	7.8	9.3	331.3	85.1	24.97		63
58	C7	7.8	9.3	331.3	141.1	17.17		63
59	C6DC adipoyl	7.8	9.3	403	85	28		77
50	C6DC adipoyl	7.8	9.3	403	129	25		77
51	C6DC 3-methylg		9.3	403	85	28		78
52	C6DC 3-methylg	7.8	9.3	403	129	27		78
52	CBUC 3-metnyiç CB:1	8	9.5	403	85.2	25		62
54	C8:1	8	9.5	343.3	141.1	17		62
55	C8	8	9.5	345.3	85.1	26		67
56	CB	8	9.5	345.3	141.1	17		67
57	C8-D3	8	9.5	348.3	85.05	23.96		64
58	C10:2	8	9.5	369.3	85	25		66
59	C10:2	8	9.5	369.3	141	19		66
70	C100H	8	9.5	389	85	27		74
71	C100H	8	9.5	389	145	22		74
72	C12:1-OH	8	10	415.3	85	29		76
3	C12:1-OH	8	10	415.3	145	21		76
4	C8DC suberoyl	8	9.5	431.4	85.1	32		82
5	C8DC suberoyl	8	9.5	431.4	157.2	26		82
6	C12DC	8	11	486	85	33		90
7	C12DC	8	11	486	213	26		90
8	C10:1	8.5	10	371.4	85.1	25		64
9	C10:1	8.5	10	371.4	141.1	18		64
10	C10	8.5	10	373.3	85.1	26		64
1	C10	8.5	10	373.3	141.1	19		64
12	C12OH	8.5	10	417.3	85	29		76
13	C120H	8.5	10	417.3	144.9	21		76
14	C14:1-OH	8.5	10	443.4	85	29		80
15	C14:1-OH	8.5	10	443.4	141	22		80
6	C10DC	8.5	10	458.4	85.1	33		101
17	C10DC	8.5	10	458.4	185.1	26		101
8	C16:1-OH	8.5	10.5	470.3	85	33		88
19	C16:1-OH	8.5	10.5	470.3	141	24		88
ю	C18:2-OH	8.5	10.8	496.3	85	30		84
1	C18:2-OH	8.5	10.8	496.3	141	23		84
12	C12:1	8.8	10.3	399.4	85.1	26		72
12	C12:1	8.8	10.3	399.4	141.1	20		72
13	C12:1 C12	8.8	10.3	400.4	85	20		72
15	C12 C12	8.8						
15 16	C12 C12-D9	8.8	10.3	400.4	141.1	19 28		79

### Mass spectrometry source conditions

Source Properties	
Ion Source Type	H-ESI *
Spray Voltage	Static *
Positive lon (V)	3250
Negative lon (V)	3600
Current LC Flow (µL/min)	Get Defaults
Sheath Gas (Arb)	60
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
lon Transfer Tube Temp (°C)	325
Vaporizer Temp (°C)	270

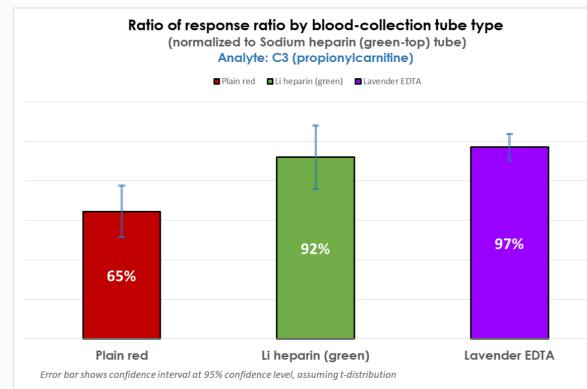


### **Reagents & sample** preparation

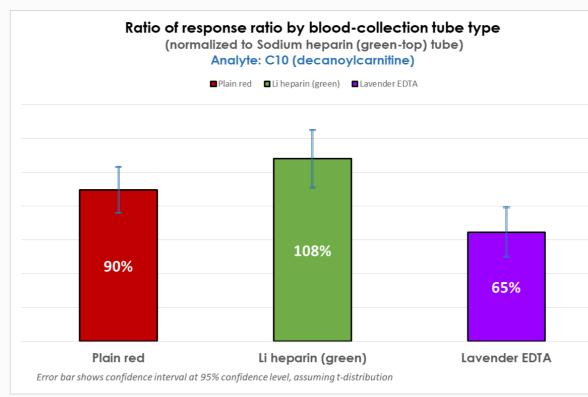
- **Reagents:**  Isotope-labeled internal standards: NSK-B-1 & NSK-B-G1-1, Cambridge Isotope Laboratories, Inc. Tewksbury, Massachusetts. USA
- Solvents: acetonitrile, methanol, Fisher Scientific, Waltham, Massachusetts, USA
- Hydrolysis reagent: potassium hydroxide, Sigma-Aldrich, St Louis, Missouri, USA
- pH-modifier in mobile phase: formic acid, Honeywell, Charlotte, North Carolina, USA
- Derivatization reagent: 3N HCl in n-butanol, Regis Technologies, Morton Grove, Illinois, USA

### Sample preparation:

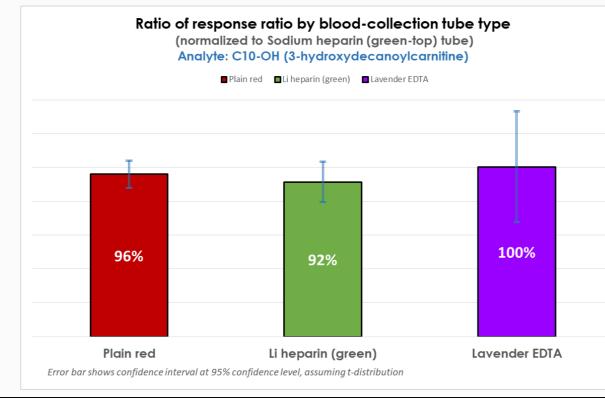
100 µL working internal standard in methanol mixed with 20 µL patient sample from each type of bloodcollection tube, vortexed sufficiently and centrifuged for 10 min at 13000 rpm (11337 xg), 80 µL supernatant is dried down, reconstituted in 100 µL 3N HCl in butanol, heated for 65°C for 15 min, dried down and reconstituted in 75 µL 60% acetonitrile:CLRW (v:v), and in 75 µL CLRW, injected on LC-MS



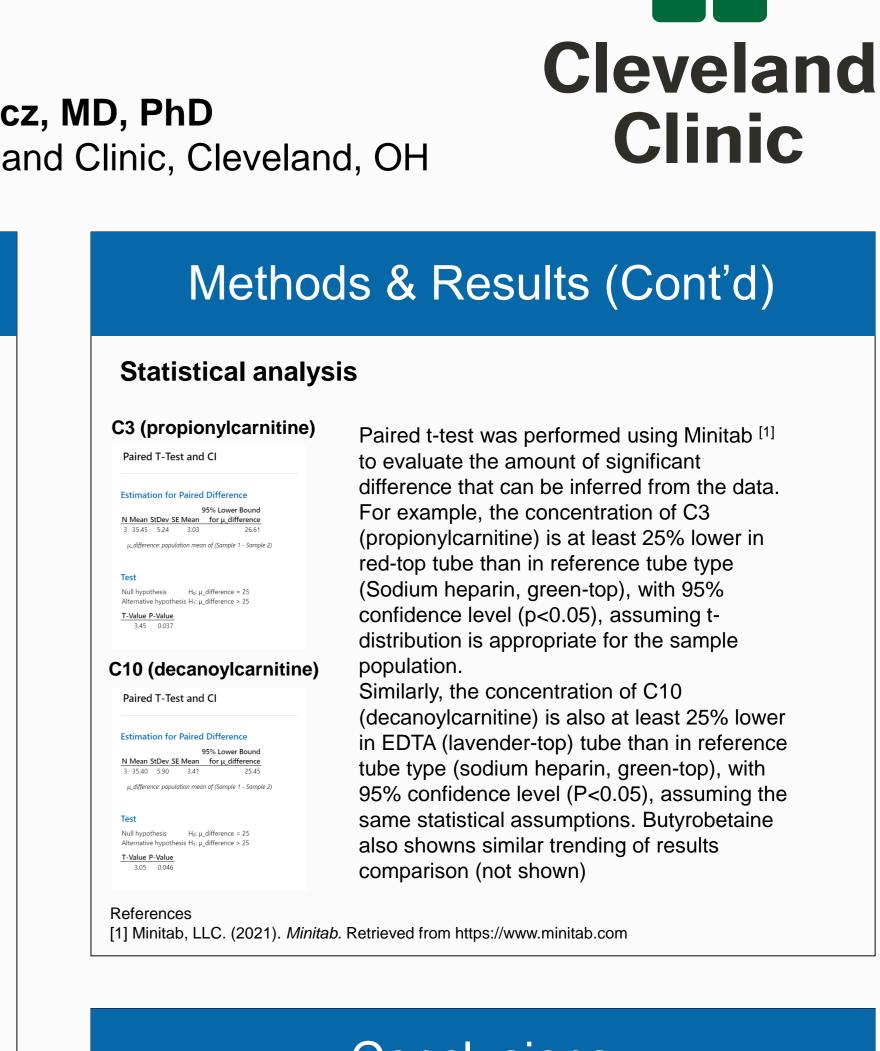
## EDTA lavender-top tube shows significantly lower results for C10



### Control: C10-OH results are not significantly impacted by the studied blood collection tube types



### Plain red-top tube shows significantly lower results for C3



# Conclusions

At least Three of 50+ measured analytes showed significant (>25-30%) matrix effects. Butyrobetaine was ~30-75% higher in heparinized plasma than in serum or EDTA anticoagulated plasma. Decanoylcarnitine was ~25-30% higher in heparinized plasma or serum compared to EDTA anticoagulated plasma. Propionylcarnitine was about 25-35% lower when measured in serum than the other tube types. When such differences straddle medical decision cut-offs, diagnostic decisions can be impacted; this may be particularly relevant for mild and late-onset forms of inherited metabolic disorders. These data suggest the utility of using matrix-specific reference intervals for those analytes that differ significantly between tube types.